

Application Serial No. 09/560,475

Atty. Docket No. 028870-178

*18/Declaration  
1.132*Patent  
Attorney's Docket No. 028870-178

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	
David C. Greenspan	)	Group Art Unit: 1615
Application No.: 09/560,475	)	Examiner: Amy E. Pulliam
Filed: April 28, 2000	)	Confirmation No.: 3797
For: Anti-Inflammatory Bioactive Glass Particulates	)	
	)	
	)	
	)	

## DECLARATION UNDER 37 C.F.R. §.1.132

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, David C. Greenspan, hereby state as follows:

**EDUCATION AND BACKGROUND**

1. I am an inventor in the above-identified application. I hold a B.S. in Glass Science from Alfred University (1972) and a Ph.D. in Materials Science from the University of Florida (1977). My Ph.D. thesis involved the development of bioactive glasses as coatings for orthopedic devices. I have over 35 publications in the area of bioactive glass research, and I hold 19 patents involving the use of bioactive glasses in the area of medical devices, including the areas of wound healing, bone regeneration and treatment of various oral health issues. I have worked in the field of biomaterials for most of the past 25 years.

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**PRODUCTION OF TNF- $\alpha$** 

2. The attached exhibit A lists TNF- $\alpha$  proteins in the Entrez search and retrieval system of the National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health database of protein sequences.<sup>1</sup> In a search of this database for "tnf alpha" in the protein name, one *homo sapiens* TNF- $\alpha$  protein was identified, indicating that there is only one *homo sapiens* TNF- $\alpha$  protein in this exhaustive collection of databases.

3. The attached exhibit B, pages 677-679 from "Immunobiology" by Janeway *et al.*, is an appendix listing cytokines, cells which produce these cytokines, and their actions. On page 678, in the TNF family, the chart states that TNF- $\alpha$  is produced by macrophages, NK cells, and T cells in local inflammation and endothelial activation. At sites of inflammation throughout the body, TNF- $\alpha$  is produced by macrophages, NK cells, and T cells.

4. The claims define a method for minimizing the production of TNF- $\alpha$  caused by an inflammatory response in a patient comprising administering locally a locally effective TNF- $\alpha$  lowering amount of bioactive glass particles with a size less than about 20  $\mu\text{m}$  to the patient. Example 2 describes that, upon intraperitoneal administration of bioactive glass to mice, IL-6 concentrations were increased, whereas the bioactive glass did not induce TNF- $\alpha$ . Example 2 further shows that peritoneal TNF- $\alpha$  was reduced in response to LPS by pretreatment with bioactive glass as claimed.

5. There is only one *homo sapiens* TNF- $\alpha$  protein, according to the Entrez search and retrieval system of the National Center for Biotechnology Information,

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<sup>1</sup> The protein entries in the Entrez search and retrieval system have been compiled from a variety of sources, including SwissProt, PIR, PRF, PDB, and translations from annotated coding regions

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National Library of Medicine, National Institutes of Health database of protein sequences. At sites of inflammation throughout the body, TNF- $\alpha$  is produced by macrophages, NK cells, and T cells. These cells which produce the TNF- $\alpha$  at one site of inflammation are the same cells which produce the TNF- $\alpha$  at another site of inflammation. In view thereof, the reduction of TNF- $\alpha$  upon administration of bioactive glass particles as shown in Example 2 in the peritoneal cavity will take place at any site in the patient.

**THE BOSETTI ET AL. ARTICLE**

6. I have read and understood the article entitled "Interaction of Bioactive Glasses with Peritoneal Macrophages and Monocytes *in vitro*", Bosetti et al., *Journal of Biomedical Materials Research*, 60(1): 79-85 (2002). Moreover, I am familiar with the bioactive glass material described in the Bosetti et al. article. Additionally, I have experience with a wide variety of sizes of particles of bioactive glass material. Upon information and belief, particles of the size claimed in present application, less than about 20 microns, will remain in the body at the site of administration less than three days, being resorbed into the body prior to that time. This is supported by the article in *Shock*, 17(2):135-138 (2002) submitted with Applicants' Amendment dated October 24, 2002.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under

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in GenBank and RefSeq. The Entrez search and retrieval system is considered an authoritative resource by those of skill in the art.

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Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name:   
David C. Greenspan

Date: June 10, 2003

06/11/2003 16:01 FAX 919 941 1515

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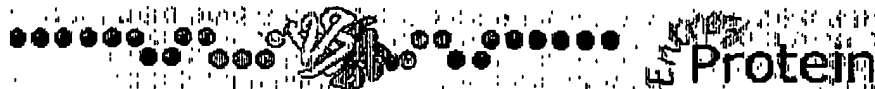
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80000 SERIES  
30% P.C.W.



Exhibit A

Received from <919 941 1515> at 6/11/03 3:55:46 PM [Eastern Daylight Time]



PubMed

Nucleotide

Protein

Genome

Structure

PMC

Taxonomy

OMIM

8c

Search Protein

for tnf alpha

Go Clear

☒ Limits

Preview/Index

History

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Details

About Entrez

Entrez Protein

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Field: Protein Name

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One page.

Batch Entrez: Upload a  
file of GI or accession  
numbers to retrieve  
sequences

Check sequence  
revision history

How to create WWW  
links to Entrez

LinkOut

Cubby

Related resources  
BLAST

Reference sequence project

LocusLink

Clusters of orthologous groups

Protein reviews on the web

☐ 1: [NP\\_038721](#)

BLink, Domains, Links

tumor necrosis factor alpha; tumor necrosis factor-alpha; TNF alpha [Mus  
musculus]  
gi|7305585|ref|NP\_038721.1|[7305585]

☐ 2: [AAL26919](#)

BLink, Domains, Links

TNF-alpha [Canis familiaris]  
gi|16588855|gb|AAL26919.1|AF327899\_1|[16588855]

☐ 3: [JU0029](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - rat  
gi|92931|pir|JU0029[92931]

☐ 4: [A25451](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - rabbit  
gi|90165|pir|A25451[90165]

☐ 5: [JH0529](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - sheep  
gi|89907|pir|JH0529[89907]

☐ 6: [S06192](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - goat (fragment)  
gi|89839|pir|S06192[89839]

☐ 7: [JQ1344](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - horse  
gi|89136|pir|JQ1344[89136]

☐ 8: [QWMSN](#)

BLink, Domains, Links

tumor necrosis factor alpha precursor - mouse  
gi|69406|pir|QWMSN[69406]

☐ 9: [CAA26669](#)

BLink, Domains, Links

TNF-alpha [Homo sapiens]  
gi|37210|emb|CAA26669.1|[37210]

- ☐ **10: AAF87741** BLink, Domains, Links  
 tumor necrosis factor alpha; TNF alpha [Capra hircus]  
 gi|9454281|gb|AAF87741.1|[9454281]
- ☐ **11: BAA13130** BLink, Domains, Links  
 TNF-alpha [Capra hircus]  
 gi|1483165|dbj|BAA13130.1|[1483165]
- ☐ **12: AAB49506** BLink, Domains, Links  
 tumor necrosis factor alpha; TNF-alpha [Trichosurus vulpecula]  
 gi|1881812|gb|AAB49506.1||bbm|391719|bbs|179895[1881812]
- ☐ **13: AAB32391** BLink, Domains, Links  
 tumor necrosis factor alpha; TNF-alpha [Canis familiaris]  
 gi|802045|gb|AAB32391.1||bbm|354002|bbs|157482[802045]
- ☐ **14: CAA68530** BLink, Domains, Links  
 tnfr-alpha [Mus musculus]  
 gi|54832|emb|CAA68530.1|[54832]

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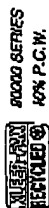


Exhibit B

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